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PARTIAL PURIFICATION AND CHARACTERIZATION OF RESTRICTION ENDONUCLEASE FROM *NEISSERIA MENINGITIDIS* (U)

by

A. Rashid Bhatti* and Richard Sparling†

Project No. 16A10

December 1983

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ABSTRACT

A restriction endonuclease, NmeI, present in *Neisseria meningitidis* DRES W34 was studied. The enzyme was partially purified by passing through a blue 2 cross-linked agarose column; no contaminating nucleases remained detectable. This enzyme cleaved phage λ, adenovirus type 2 (Ad 2) and Φ × 174 DNA but did not cleave simian virus 40 (SV40) DNA. It had an absolute requirement for Mg^{2+} for its activity and was inhibited by high concentrations of NaCl or $MgCl_2$. NmeI activity was completely abolished after one hour of incubation at 65°C. S-adenosyl-L-methionine and ATP had no effect on its activity suggesting that NmeI is a type II restriction endonuclease enzyme.

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INTRODUCTION

Restriction and modification systems have been extensively studied in a number of bacterial species. A restriction endonuclease recognizes a specific nucleotide sequence in double stranded DNA and cleaves both strands, fragmenting the DNA. These are part of the restriction-modification system which was first described by Luria and Human (7). This system maintains the integrity of a bacterial genome and protects the cell from invasion by foreign DNA. Three different types of restriction-modifications have been recognized (9, 11, 15). Type I restriction endonucleases cleave DNA at non-specific sites far removed from the recognition site and require ATP, S-adenosyl-L-methionine and Mg^{2+} for their activity. Type III restriction endonucleases require ATP

and Mg^{2+} for their activity and cleave DNA 24 – 26 base pairs from the 3′ side of the recognition site. The recognition sites recognized by type I and type III enzymes are nonpalindromic. Type II enzymes recognize palindromic sequences, require only Mg^{2+} for their activity, and cleave substrate DNA at a specific site within or at the end of the recognition sequence. The first type II restriction endonuclease to be discovered was the Hind II enzyme (14); since then, over 200 enzymes of this type have been reported (12). These enzymes have found wide application in the analysis and restructuring of DNA molecules and also provide convenient systems for studies on the interaction between proteins and specific sequences in nucleic acids since they require only Mg^{2+} as a cofactor (12). We report here the partial purification and characterization of a type II restriction endonuclease from *N. meningitidis* DRES W34. Hereafter, this enzyme activity will be referred to as NmeI following the nomenclature suggested by Smith and Nathan (13).

MATERIALS AND METHODS

Organism and Culture Conditions

N. meningitidis strain, DRES W34 was cultivated in one liter of brain heart infusion broth in 6 L Erlenmeyer flasks. Growth was carried out in an gyratory shaker incubator for 16 h with shaking at 35°C in an atmosphere of 5% $C0_2$ in air (3). Cells were harvested at 4°C by centrifugation at 15,000 × g for 10 min.

Preparation of Cell-Free Extract

The cell pellet was suspended in 10 mL of Tris-HCl buffer pH 7.6 (Tris, 20 mM, $MgCl_2$, 10 mM and dithiothreitol, 10 mM; buffer A) and ultrasonically disrupted by Soniprep 150 sonicator using 20 × 10 sec burst on ice. Cell free extract (CFE) was obtained by centrifugation at 100,000 × g for 1 h. To the CFE, glycerol was added to a final concentration of 10% and stored at −70°C till further use.

Purification of Enzyme

NmeI was partially purified according to the method of Baksi *et al.*, (1). The CFE was passed through a column (0.9 × 9 cm) packed with reactive blue 2 cross-

linked agarose (Sigma) previously washed with buffer A. The column was washed with 15 mL of buffer A to remove nucleic acid and non-binding proteins. The enzyme was eluted by single step elution with 0.5 M NaCl (30 mL) in buffer A. Fractions (2.5 mL) were collected on an automatic fraction collector and protein concentration was monitored by measuring the absorbance at 280 nm. The fractions with the highest protein concentration (fractions 5 – 10) were pooled and dialysed against buffer A in the presence of glycerol (10 % v/v). The dialysate was stored at –70°C.

Enzyme Assay

The enzyme activity of NmeI was assayed by agarose gel electrophoresis. The standard assay mixture contained 5 μL of substrate DNA (λ, simian virus 40 (SV40), adenovirus type 2 (Ad 2), Φ × 174 (Sigma) diluted to a unit activity per mL at 260 nm) in buffer A, and 5 μL of BSA (0.4 μg/mL). The reaction was initiated by adding 35 μL of enzyme sample and the reaction mixture was incubated at 37°C for 1 h. The reaction was stopped by adding 5 μL of a solution containing 7% sodium dodecyl sulfate (SDS), 0.2 M ethylene diamine tetra acetic acid (EDTA), 25% Ficol 40,000 to which phenol red was added as an indicator. The whole assay reaction mixture was used for electrophoresis.

Gel Electrophoresis

Agarose gel electrophoresis was performed as follows: gel was prepared by 1.4% Seakam (Sea Plaque) agarose in Tris-acetate buffer pH 7.9 which contained Tris 40 mM, sodium acetate 5 mM and EDTA 1 mM (buffer B). Electrophoresis was carried out in a vertical slab gel apparatus (Bio-Rad), using buffer B usually for 1.5 h at 100 V until the dye migrated 1 inch from the bottom of the gel. The gel was then stained with ethidium bromide (0.2 μg/mL) for 1 h. DNA fragments were visualized with a short wave U.V. trans-illuminator. Photographs were taken with a Polaroid type 55 P/N film exposed through a red filter.

RESULTS AND DISCUSSION

Restriction endonucleases have been found in most bacterial genera. Different types of restriction enzyme activity present in a variety of microorganism have been

reviewed by Roberts (11). A cell free extract obtained from *N. meningitidis* when incubated with λ DNA and analyzed by agarose gel electrophoresis did not give a clean fragmentation pattern. This effect may have been due to the presence of other exo- and endo-nucleases in the CFE (1) which made the determination of restriction endonuclease activity impossible at this point. Therefore, to determine whether there is any restriction endonuclease activity present in *N. meningitidis*, the enzyme was purified according to the method for rapid purifications of restriction endonucleases, described by Baksi *et al.*, (1). The enzyme was eluted from the column with 0.5 M NaCl in buffer A. Very little restriction endonuclease activity was observed in the eluant. However, subsequent dialysis against Buffer A (as mentioned in Material and Methods) did lead to the restoration of enzyme activity as shown by fragmentation of λ DNA (Figure 1, Channel 1). The enzyme so purified was free of contaminating nucleic acid and other nucleases (1) and was sufficently concentrated for direct, specific DNA hydrolysis. Inhibition of NmeI by NaCl was further studied. To find the concentrations of NaCl that would inhibit restriction enzyme activity, samples were assayed in the presence of different concentrations of NaCl (data not shown). NmeI retained full enzyme activity at NaCl concentrations of up to 0.2 M while complete inactivation was seen at 0.4 M NaCl. Similar inhibitory effects were observed with Bsp obtained from *Bacillus sphaericus* as reported by Knocz, Kiss and Venetainer (4). These results suggest that NmeI is reversibly inhibited by the high concentration of NaCl.

All Type II restriction endonucleases require Mg^{2+} as an essential cofactor (9, 11). To demonstrate that NmeI required Mg^{2+} as a cofactor for its enzyme activity, a sample of NmeI was exhaustively dialyzed in Buffer A without $MgCl_2$ and tested for restriction endonuclease activity. The effect of Mg^{2+} is illustrated in Figure 1. NmeI gave maximum restriction endonuclease activity at a $MgCl_2$ concentration of 0.01 M as shown by the series of low molecular weight bands channels 1 and 2 of Figure 1. Concentrations of $MgCl_2$ above 0.02 M have an inhibitory effect on the restriction endonuclease activity of NmeI. This inhibitory effect is clearly evident from the fragmention pattern of intermediate mobilities obtained by the partial cleavage of λ DNA (Channels 3 – 5). In the absence of Mg^{2+}, limited restriction endonuclease activity was observed (Channel 5). These results demonstrate that NmeI has an absolute and a precise requirement for Mg^{2+} for its optimal restriction enzyme activity. EDTA, an inhibitor of metalo-enzymes at low concentration, completely inhibited the enzyme activity of NmeI as indicated by the uncleaved λ DNA band at the top of the gel

(Channel 7). Recently, Barton, Basil and Parananivethan (2) have reported that EcoR1, a type II restriction enzyme from *Escherichia coli* is a zinc-metallo-enzyme. Our results on the effect of EDTA also suggest that NmeI is a type II metallo-enzyme. Polisky *et al.*, (10) have reported a relaxing of specificity of the recognition sites of EcoRI at higher ionic strengths which could be the case here as well. Alterations in the rates of activity at higher $MgCl_2$, and indeed at higher ionic strengths in general (as also demonstrated by the NaCl inhibition effect explained earlier), were also reported by Maxwell and Halford (8) for SalGI and for NciI by Watson, Zuker, Martin and Visentin (16).

In another set of experiments to check for the presence of a Type I and Type III restriction enzyme in the sample, ATP and S-adenosyl-L-methionine (0.4 mM and 0.04 μM final concentrations, respectively), were also added to the assay mixture. However, such an addition did not alter the fragmentation of λ DNA. Therefore, both ATP and S-adenosyl-L-methionine are not required for this enzyme activation. These results suggest that NmeI activity is a typical type II restriction endonuclease.

The effect of high temperature on the stability of NmeI is presented in Figure 2. Enzyme samples were incubated at different temperatures for 30 min before assaying. Samples incubated at 37°C or below showed unaltered enzyme activity with λ DNA while there was little enzyme activity observed in samples incubated at 45°C and 55°C. The enzyme was completely inactivated after incubation at 65°C. These results are comparable to those reported for NgoII (6). Samples kept at −70°C in 10% glycerol are stable for over a month.

In addition to these various properties, it was important to establish the fragmentation pattern of NmeI on different DNA substrates. The results in Figure 3 show that NmeI cleaved λ DNA into at least 18 fragments, Φ × 174 was cleaved once and there was no noticeable cleavage of SV40. NmeI also cleaved Ad 2 DNA into 8 fragments (data not shown). The cleavage patterns described here are different from those reported by Clanton *et al.* for NgoII, an isoschizomer of HaeIII (5) and from NgoI an isoschizomer of HaeII (7). To check that NmeI was not an isoschizomer of HaeIII, NmeI and HaeIII were mixed and used to digest λ DNA. Figure 4 shows the difference between the NmeI and HaeIII. The double digest of both enzymes on λ DNA gave a fragmentation pattern dissimilar to that produced by either enzyme alone, suggesting that NmeI was indeed not an isoschizomer of restriction endonucleases from

N. gonorrhoeae and *Haemophilus aegyptius*. Similar to NmeI restriction endonuclease NciI from *N. cineria* (16) cleaved Φ × 174 DNA at one site but did not cleave SV40 DNA. NciI cleaved Ad 2 DNA at more than 15 different sites. However, in the absence of cleavage activity of NmeI toward Ad 2 DNA it is difficult to compare these two enzymes at this time.

In conclusion, this is the first report of a restriction enzyme in *N. meningtidis*. This endonuclease, NmeI belongs to the family of type II restriction endonucleases.

REFERENCES

1. Baksi, K., Rogerson, D.L. and Rushizky G.W. (1978). Rapid, single step purification of restriction endonuclease on ciliacorn blue FWGA-Agarose. *Biochem.* **17**: 4136 – 4139.

2. Barton, K.J., Basil, L.A. and Paranwithana, S.R. (1982). The presence of zinc in the restriction enzyme EcoRI. *J. Biol. Chem.* **258**: 7911 – 7914.

3. Bhatti, A.R., O'Hara, K., White, L.A. and Bryan, L.E. (1981). Presence of plasmid deoxyribonucleic acid in *Neisseria meningitidis*. *FEMS Microbiol. Letters* **10**: 157 – 177.

4. Koncz, C., Kiss, A. and Venetainer, P. (1978). Biochemical characterization of restriction modification system of *Bacillus sphaericus*. *Eur. J. Biochem.* **89**: 523 – 529.

5. Clanton, D.J., Woodward, J.M. and Miller, R.V. (1978). Identification of a new sequence-specific endonuclease, NgoII, from *Neisseria gonorrhoeae*. *J. Bacteriol.* **135**: 270 – 273.

6. Clanton, D.J., Woodward, J.M. and Miller, R.V. (1979). NgoII, a restriction endonuclease from *Neisseria gonorrhoeae*. *J. Bacteriol.* **137**: 1299 – 1307.

7. Luria, S.E. and Human, M.L. (1952). A non-hereditary, host induced variation of bacterial viruses. *J. Bacteriol.* **64**: 557 – 569.

8. Maxwell, A. and Halford, S.E. (1982). The SalGI restriction endonuclease purification and properties. *Biochem. J.* **203**: 77 – 84.

9. Modrich, P. (1979). Structures and mechanisms of DNA restriction and modification enzymes. *Quart. Rev. Biophys.* **12**: 315 – 369.

10. Polisky, B. Greene, P., Garfin, D.E., McCarthy, B.J. Goodman, H.M. and Boyer, H.W. (1975). Specificity of substrate recognition by EcoRI restriction endonuclease. *Proc. Nat. Acad. Sci.* **72**: 3310 – 3314.

REFERENCES (Cont'd)

11. Roberts, R.J. (1976). Restriction endonucleases. *CRC Crit. Rev. Biochem.* **3**: 123 – 164.

12. Roberts, R.J. (1981). Restriction and modification enzymes and their recognition sequences. *Nucleic Acid. Res.* **9**: 75 – 96.

13. Smith, H.O. and Nathans, D. (1973). A suggested nomenclature for bacteria host modification and restriction systems and their enzymes. *J. Mol. Biol.* **81**: 419 – 423.

14. Smith, H.O. and Wilcox, K.W. (1970). A restriction enzymes from *Hemophillus influenzae*. 1. Purification and general properties. *J. Mol. Biol.* **51**: 379 – 391.

15. Yuan, R. (1981). Structure and mechanism of multifunctional restriction endonucleases. *Ann. Rev. Biochem.* **50**: 285 – 315.

16. Watson, R., Zuker, M. Martin, S.M. and Visentin, L.P. (1980). A new site-specific endonuclease from *Neisseria cineria*. *FEBS Letters* **118**: 47 – 50.

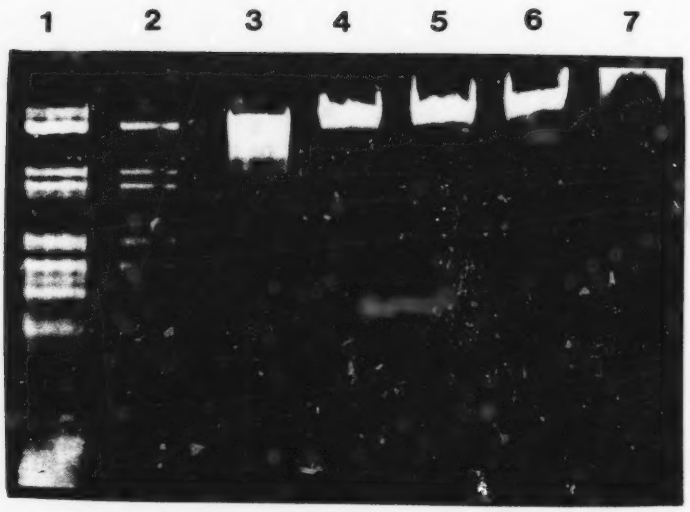

FIGURE 1

The effect of Mg^{2+} on *NmeI* enzyme activity. Enzyme sample was dialysed thoroughly against buffer A containing 10% glycerol without Mg^{2+}. Enzyme activity of dialysed *NmeI* was assayed in the presence of: 1. 5 mM $MgCl_2$; 2. 10 mM $MgCl_2$; 3. 20 mM $MgCl_2$; 4. 50 mM $MgCl_2$; 5. 100 mM $MgCl_2$; 6. No $MgCl_2$; 7. 1 mM EDTA, respectively, and λ DNA was used as substrate.

FIGURE 2

Agarose gel showing thermal stability of *NmeI* at various temperatures. The enzyme samples 2 - 7 were incubated at respective temperatures 25°, 37°, 45°, 55° and 65°C for 30 min and assayed for residual enzyme activity as described in Materials and Methods. Phage λ DNA was used as substrate. Control sample 1 was kept at -70°C and thawed immediately before assay.

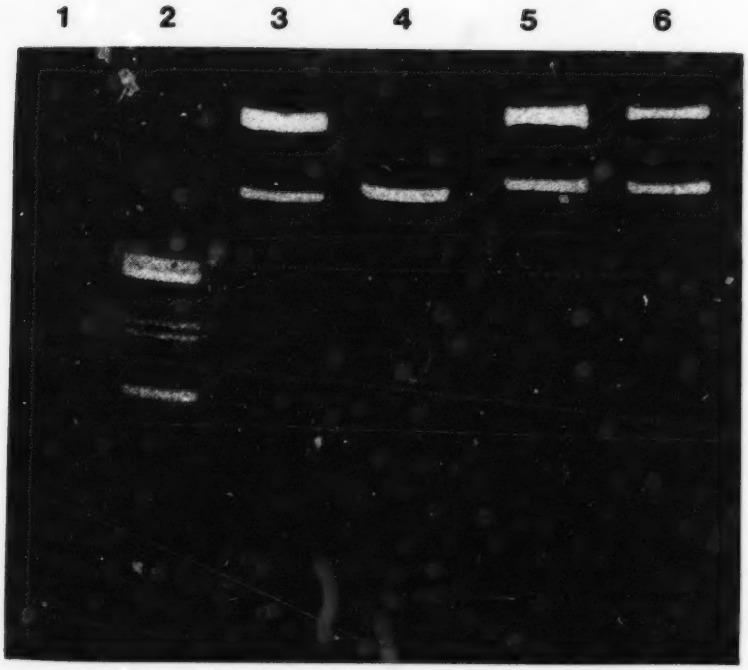

FIGURE 3

Agarose gel showing the fragmentation patterns caused by *NmeI* on different substrate DNA's. Channels 1, 3 and 5 represent controls for phage λ, ϕ × 174 and SV40 DNA while channels 2, 4 and 6 represent respective DNA after treatment with *NmeI*.

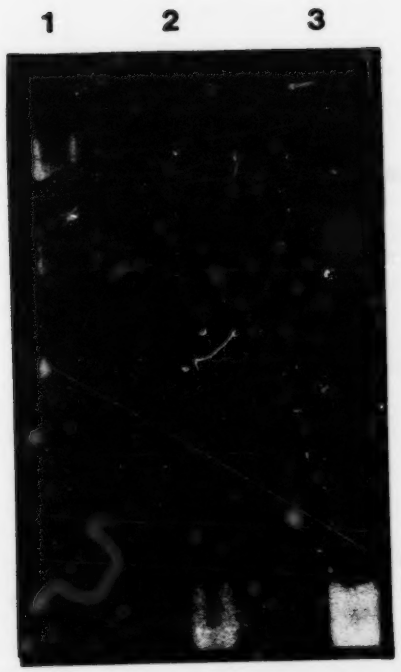

FIGURE 4

Agarose gel of fragmentation patterns of phage λ DNA after digestion with *NmeI* and *HaeIII*: 1. *NmeI*, 2. a mixture of *NmeI* and *HaeIII*, 3. *HaeIII*.

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